




UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460


OFFICE OF CHEMICAL SAFETY  
AND POLLUTION PREVENTION

**MEMORANDUM**

**AUG 07 2014**

**SUBJECT:** Deficiency memorandum for the FIFRA Section 3 registration of *Isaria* (formerly, *Paecilomyces*) *fumosoroseus* FE 9901 (EPA File Symbols 73314-6, 73314-7) Decision Nos. 481554, 481555; DP# 417625; Submission Nos. 938912, 938913; E-Sub #s: 4568, 4569

**FROM:** Gail Tomimatsu, Ph.D., Plant Pathologist   
Microbial Pesticides Branch  
Biopesticides and Pollution Prevention Division, 7511P

**THRU:** Shannon Borges, Lead Biologist   
Microbial Pesticides Branch  
Biopesticides and Pollution Prevention Division, 7511P

**TO:** Jeannine Kausch, Regulatory Action Leader  
Microbial Pesticides Branch  
Biopesticides and Pollution Prevention Division, 7511P

Novozymes has applied for FIFRA Section 3 registration of *Isaria* (formerly, *Paecilomyces*) *fumosoroseus* FE 9901 for control of insect pests on outdoor and greenhouse terrestrial food, turf and ornamental crops for commercial and residential purposes. Labeled applications include foliar, soil drench, hydroponic and chemigation treatments with repeat applications at 15-day intervals for control of whitefly, aphids, thrips, psyllids, mealybugs and fungus gnats. The frequency of application is not clear on the present label. The EP NoFly (EPA Reg. No. 73314-6) is a wettable powder formulation with 18% a.i., and includes fermentation solids and blastospores. Since *Isaria fumosoroseus* FE9901 is currently registered for indoor uses only, the manufacturer submitted an application for a label amendment to include the outdoor uses.

Studies and scientific rationales were submitted to partially satisfy data requirements for nontarget organisms as published in 40 CFR § 158.2150. Evaluations of these studies (data evaluation records, DERs) were conducted for the purposes of characterizing potential toxic or pathogenic hazards to nontarget organisms, and for regulatory support of amending the current registration to outdoor applications. With the exceptions of avian oral toxicity, nontarget insect, and honeybee data requirements (OPPTS Guidelines 885.4050, 885.4340, and 885.4380 respectively), all nontarget organism data requirements are complete. Due to the deficiencies outlined below, there is uncertainty concerning the potential toxic spectrum and environmental stability of beauverolides to nontarget organisms, especially to avian, and insect (including honeybees) species.

**Recommendations:** In order to support the Section 3 registration for this new a.i., an acute avian oral toxicity study must be submitted for review. Precautionary and mitigating label language to protect honeybees and nontarget insects are required on end-use products. The frequency of application must be clarified on end-use label(s).

**Deficiencies:** Notable deficiencies are itemized below. Detailed information and analyses are provided in the attached DERs.

- (1) *An avian oral toxicity study must be submitted* for review. The submitted rationale for the avian data requirement was classified as Supplemental for the infectivity/pathogenicity component of the data requirement and Unacceptable for the toxicity component. There were no comments in the submitted rationale concerning infected (intoxicated) insects or plant surfaces that would be available to avian and wild mammal predators. Treated crops (e.g., seedlings in-furrow) and insects are available for consumption, such that nontarget terrestrial wildlife would be primarily exposed to mycotoxins via the diet. There were no submitted data or literature to confirm lack of concern for toxicity by *Isaria fumosoroseus* to avian species. Secondary metabolites and potential toxins of concern were identified in production batches of NoFLY™ Technical; and although the quantities were in the low ppm to ppb range, the toxicity of these metabolites have not been fully evaluated upon exposure to nontarget organisms, especially avian species. Significant fish mortalities and significant reduced daphnid reproduction (fertility and/or fecundity) were noted in submitted aquatic studies (MRIDs 49118303 and 49118304) for test animals exposed to *Isaria fumosoroseus* FE9901 compared to untreated controls. It is recommended that an avian oral toxicity study be conducted in accordance with the following OCSPP guidelines: (a) OCSPP Guideline 850.2100 for guidance on appropriate experimental design and reporting, and OCSPP Guideline 885.4020 for testing appropriate levels or doses of the microbial pesticide. BPPD strongly recommends the registrant consult with Agency staff before testing.
- (2) Results of the non-GLP, non-guideline honeybee test were inconclusive, and therefore the honeybee data requirement is incomplete. Precautionary and mitigating label language to protect honeybees are required on end-use products until submission and review of an OPPTS guideline honeybee study.
- (3) Non-GLP, non-guideline studies conducted on certain nontarget insects (Orders Hemiptera, Homoptera and Hymenoptera) were classified as Supplemental: (1) only summaries of six laboratory tests and one semi-field test were reported; and (2) no biological endpoints (i.e., NOEC, LC50 values) were reported, and adverse effects were observed in some of the tests on predatory bugs. The submitted studies determined the effects of *Isaria* (formerly *Paecilomyces*) *fumosoroseus* on mortality and reduction in parasitism of whitefly parasitoids and predators. The nontarget insect data requirement is incomplete. Since the product has intended insecticidal claims, precautionary and mitigating label language to protect beneficial insects are required on end-use products until submission and review of OCSPP guideline nontarget insect studies.

- (4) *Certificates of Analysis must be revised for the record.* The Certificates of Analysis for the test materials and the sterile filtrate controls in the aquatic studies were incomplete and must be clarified for the record. The term “potency” must be clarified, since it is expressed in units of viability. Additionally, the manufacturer must explain how viability is determined, and clarified with citation to an SOP if provided in a previously submitted study volume. A short description of the microbiology methodology and how the “viable” microbial populations were determined (quantified) for the test material must be included. Details are provided in the associative Data Evaluation Records (DER). Certificates of Analysis were not submitted for the non-GLP nontarget insect and honeybee studies (MRIDs 49118305 and 47970501, respectively).



## DATA EVALUATION RECORD

EPA Primary Reviewer: Gail Tomimatsu, Ph.D. *gt*

AUG 07 2014

EPA Secondary Reviewer: Shannon Borges, Team Leader *SB*

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**STUDY TYPE:** Waiver Requests:

Avian Oral, Tier I (OCSPP 885.4050)  
Avian Inhalation Test, Tier I (OCSPP 885.4100)  
Wild Mammal Testing, Tier I (OCSPP 885.4150)  
Estuarine and Marine Animal Testing, Tier I (OCSPP 885.4280)  
Nontarget Plant Studies, Tier I (OCSPP 885.4300)

**MRID NO:** 49118302

**DECISION NO:** 481554; 481555

**DP BARCODE:** 417625; 417629

**TEST MATERIAL:** *Paecilomyces fumosoroseus* FE9901 (TGAI)

**PROJECT STUDY NO:** NoFly-20130514-2

**SPONSOR:** Novozymes BioAg, Inc., 13100 W. Lisbon Road, Suite 600,  
Brookfield, WI 53005

**TESTING FACILITY:** Not applicable

**TITLE OF REPORT:** Response to Tier 1 Microbial Pesticide Data Requirements for  
NoFly™ Technical (EPA Reg. No. 73314-7)

**AUTHOR:** Milesen, B.E.

**STUDY COMPLETED:** June 7, 2012

**CONFIDENTIALITY**

**CLAIMS:** None.

**GOOD LABORATORY**

**PRACTICE:** A signed and dated GLP statement was provided. The study volume is a summary compilation and contains no measured data. As such, it is not subject to the requirements of 40 CFR Part 160.

**CONCLUSION and CLASSIFICATION:** Acceptable for Wild Mammal Testing, Tier 1 and Nontarget Plant Testing, Tier 1. Supplemental for the following data requirements: Avian Oral Tier I (Pathogenicity), Estuarine and Marine Animal Testing, Tier I. The rationale is Unacceptable for assessing potential toxicity to avian species. Data and results of an avian oral study must be provided to complete the risk characterization of *Isaria* (formerly, *Paecilomyces*) *fumosoroseus* FE9901 to avian species. Further details are provided in the Reviewers' Conclusions and Comments.

### Product Description

NoFly WP is an end use product for use against whitefly, aphids, thrips, psyllids, mealybugs, and fungus gnats on greenhouse and outdoor ornamentals, trees and shrubs, nursery crops, turf, and production agriculture crops. The active ingredient is 18.0% w/w *Paecilomyces fumosoroseus* FE9901, containing a minimum of  $2 \times 10^9$  cfu/g dry weight. The proposed product label (6/6/2012) states that the inert ingredients in the product are food grade. The product is currently registered (EPA Reg. No. 73314-6) for greenhouse use only.

### Waiver Requests

The registrant is requesting a waiver from the following requirements:

MRID 49118302

Avian Oral, Tier I	(OCSPP 885.4050)
Avian Inhalation Test, Tier I	(OCSPP 885.4100)
Wild Mammal Testing, Tier I	(OCSPP 885.4150)
Estuarine and Marine Animal Testing, Tier I	(OCSPP 885.4280)
Nontarget Plant Studies, Tier I	(OCSPP 885.4300)

### **Registrant's Justification**

#### **Avian Oral, Tier I**

*P. fumosoroseus* is reported to be low in toxicity to birds (Blockmans et al., 1995). No mortalities were recorded in any of the acute toxicity tests conducted, although the doses were not reported.

Pathogenicity is not expected in birds orally exposed to NoFly because *P. fumosoroseus* strain FE 9901 is not able to survive at bird body temperature. Avian body temperatures are almost always higher than those of mammals of the same weight and generally range between 38-42°C (McNab, 1966). *P. fumosoroseus* strain FE 9901 does not grow at 35°C and is killed at 37°C (Gerger, 2004).

The proposed use pattern and low application rate of the product suggest that the potential exposure to birds will be low. The proposed use pattern is a foliar spray targeting the undersides of the plant leaves, which is not expected to provide optimal conditions for product pooling and consumption by birds. The maximum application rate is two pounds/acre, and the *P. fumosoroseus* strain FE 9901 spores make up only 18% of the product. Thus, the amount of TGAI per acre would be 0.36 lb (163.3 g/A). *P. fumosoroseus* has been shown to degrade in sunlight (Fargues, et al., 1997). Rapid degradation in sunlight and warm temperatures will further reduce the potential for avian oral exposure.

Beauverolide peptide mycotoxins in the TGAI are present only at very low levels (Simek et al., 2012; MRID 49118301). The study identified the principal beauverolide peptide, beauverolide I, and 14 others in the class according to their chromatographic and mass spectral properties. Beauverolide I was determined to be present at a range of 50 to 150 µg/g. Since beauverolide I was approximately 18% of the combined beauverolide peptides, 100% of the combined peptides are well below 0.1% of the TGAI and are not of toxicological concern for avian oral exposure. No data or literature were provided to confirm lack of concern for toxicity to avian species.

#### **Avian Inhalation Test, Tier I**

*P. fumosoroseus* is reported to be low in toxicity to birds (Blockmans et al., 1995). No mortalities were recorded in any of the acute toxicity tests conducted, although the doses were not reported.

Pathogenicity is not expected in birds orally exposed via inhalation of NoFly because *P. fumosoroseus* strain FE 9901 is not able to survive at bird body temperature. Avian body temperatures are almost always higher than those of mammals of the same weight and generally range between 38-42°C (McNab, 1966). *P. fumosoroseus* strain FE 9901 does not grow at 35°C and is killed at 37°C (Gerger, 2004).

The proposed use pattern and low application rate of the product suggest that the potential exposure to birds will be low. The proposed use pattern is a foliar spray targeting the undersides

of the plant leaves, which is not expected to result in a widespread aerosol that might present significant opportunities for inhalation exposure. Additionally, *P. fumosoroseus* has been shown to degrade in sunlight (Fargues, et al., 1997). Rapid degradation in sunlight and warm temperatures will further reduce the potential for avian inhalation exposure.

Beauverolide peptide mycotoxins in the TGAI are present only at very low levels (Simek et al., 2012; MRID 49118301). The study identified the principal beauverolide peptide, beauverolide I, and 14 others in the class according to their chromatographic and mass spectral properties. Beauverolide I was determined to be present at a range of 50 to 150 µg/g. Since beauverolide I was approximately 18% of the combined beauverolide peptides, 100% of the combined peptides are well below 0.1% of the TGAI and are not of toxicological concern for avian inhalation exposure.

#### Wild Mammal Testing, Tier I

*P. fumosoroseus* strain FE 9901 was not toxic or pathogenic to rats exposed to a single gavage dose of 1 mL of the TGAI containing  $1 \times 10^8$  cfu/mL (Cerna, 2004; MRID 47792002). No pathogenicity or persistence of *P. fumosoroseus* strain FE 9901 occurred in the rats.

There is no reason to believe that studies conducted to evaluate potential human health effects from exposure to *P. fumosoroseus* strain FE 9901 would not be relevant to wild mammals. The potential exposure to wild mammals would not be unusually high based on the proposed use pattern of foliar application targeting the underside of plant leaves. In addition, *P. fumosoroseus* strain FE 9901 degrades in sunlight (Fargues, et al., 1997), which would further reduce the potential for wild mammal exposure.

#### Estuarine and Marine Animal Testing, Tier I

Exposure of the estuarine or marine environment to *P. fumosoroseus* strain FE 9901 is expected to be low due to the proposed use pattern of foliar application directed to the undersides of plant leaves at a rate of approximately 163.3 g/A. Furthermore, *P. fumosoroseus* strain FE 9901 readily degrades in sunlight (Fargues, et al., 1997), reducing the potential for it to reach estuarine or marine environments.

#### Nontarget Plant Studies, Tier I

Footnote 7 of 40 CFR 158.2150 indicates nontarget plant testing is required if the MPCA is taxonomically related to a plant pathogen. *P. fumosoroseus* strain FE 9901 is an entomopathogenic fungus that has been isolated from infected insects, and is not related to plant pathogen (Bolckmans et al., 1995).

#### Literature Cited

Blockmans, K., G. Sterk, J. Eyal, et al. 1995. PreFeRal (*Paecilomyces fumosoroseus* strain Apopka 97). A New Microbial Insecticide for the Biological Control of Whiteflies in Greenhouses. Med. Fac. Landbouww. Univ. Gent. 60/3a:707-711.

Fargues, J. M. Rougier, R. Goujet, et al. 1997. Inactivation of Conidia of *Paecilomyces fumosoroseus* by Near-Ultraviolet (UVB and UVA) and Visible Radiation. J. Invertebrate Pathology 69(1):70-78.

Gerger, R. 2004. Characterisation of the Microbial Pest Control Agent *Paecilomyces fumosoroseus* strain FE 9901. Department de Microbiologia Universitat de Barcelona. Doc J-2c. Submitted to Futureco S.L. 5-12-2004.

McNab, B.K. 1966. An Analysis of the Body Temperature of Birds. The Condor 68:47-55.

### **EPA Reviewer's Conclusion and Comments**

The taxonomic change of *Paecilomyces* to *Isaria* was submitted and reviewed (MRID 49118303) after the submission of the present study volume.

For this study volume (MRID 49118302), submitted rationale was classified as Supplemental to support the following nontarget organism data requirements: Avian Oral Testing, Tier I (Pathogenicity only); and the Estuarine and Marine Animal Testing, Tier I in this study report (MRID 49118302). The submitted rationales for the wild mammal and nontarget plant test data requirements are Acceptable. EPA reviewers disagree with the study author's rationale comments concerning "proposed use pattern of foliar application directed to the undersides of plant leaves." Directing foliar applications to the "undersides of leaves" is insufficient to minimize pesticidal exposures to avian species. The top-side of plant foliage would also be exposed to foliar sprays via ground and chemigation equipment (overhead irrigation, e.g.), and birds could be exposed via consumption of treated plants or insects. Intended insecticidal uses and exposures to nontarget organisms are not only directed foliar sprays; and include a broad range of application scenarios to soil and plant surfaces with ground and chemigation equipment, such that the top-side of plant foliage would likely be treated. The study author claims that *P. fumosoroseus* FE9901 conidia was reported to degrade under sunlight, (Fargues, et al., 1997), but did not note that the results were obtained under simulated sunlight conditions. In addition, the results varied depending on the wavelength and the exposure times; such variability would be expected under natural conditions and likely result in inconsistent insect pest control. The germination rate, viability and infectivity of conidia on treated shaded control plants remained fairly stable throughout irradiation, possibly because of the short exposure durations.

There were no comments in the submitted rationale concerning infected (intoxicated) insects or plant surfaces that would be available to avian and wild mammal predators. Treated crops (e.g., seedlings in-furrow) and insects are available for consumption, and nontarget terrestrial wildlife (avian and wild mammals) would be exposed to mycotoxins via the diet. There were no submitted data or literature to confirm lack of concern for toxicity by *Isaria fumosoroseus* to avian species. Secondary metabolites and potential toxins of concern were identified in production batches of NoFLY™ Technical; and although the quantities were in the low ppm to ppb range, the toxicity of these metabolites have not been fully evaluated upon exposure to nontarget organisms, especially avian species. It is recommended that an avian oral toxicity study be conducted in accordance with the following OCSPP guidelines: (a) OCSPP Guideline 850. 2100 for guidance on appropriate experimental design and reporting, and OCSPP Guideline 885.4020 for testing appropriate levels or doses of the microbial pesticide.

Because of the toxicities noted in the rainbow trout test (MRID 49118303), and uncertainty in the toxicity of beauverolides that may be present in the end-use product (MRID 49118301), an avian toxicity study is required to fulfill the nontarget avian oral data requirement. Information and data from other studies submitted for mammalian toxicity and acute infectivity testing (MRIDs 4772002, 4772004, 4772005 and 49044301) are sufficient to support the data requirement for Wild Mammal Testing, Tier I. The data requirement for Wild Mammal Testing is complete.

Significant and directed exposures of *Isaria* (taxonomic name change and response in MRID 49118303) *fumosoroseus* are not expected to organisms in estuarine and marine environments.

There are no reports of plant pathogenic *Isaria*; the taxonomic name change was classified Acceptable elsewhere in the Agency's documentation. The genus *Paecilomyces* has a few species that have reports of symbiotic relationships with plants [1]: *P. carneus* (Acer spp); *P. lilacinus* (insects and Carya); *P. marquandii* (Acer and Pinus) and *P. varioti* (Carya, Helianthus, Pinus). *Paecilomyces buxi* (root decline of boxwood) is currently considered as *Sesquicillium buxi* [2].

It should be noted that there was no information on the fate and behavior of toxins (i.e., beauverolides) or secondary metabolites in the environment. No information was provided to demonstrate that, under conditions of proposed uses, any toxins/secondary metabolites produced by *Isaria fumosoroseus* FE9901 will not occur in concentrations significantly higher than under natural conditions. Accordingly, additional data on the persistence, transformation and mobility of these compounds may be necessary.

#### References:

1. Farr, D.F., G. Bills, G. Chamuris, A. Rossman. 1989. Fungi on Plants and Plant Products in the United States, p. 825. 1252 pages.
2. Horst, R., editor. 2008. Wescott's Plant Disease Handbook, 7<sup>th</sup> edition, p. 501. 1317 pages

#### Literature Cited

- Bolckmans, K., G. Sterk, J. Eyal, et al. 1995. PreFeRal (*Paecilomyces fumosoroseus* strain Apopka 97). A New Microbial Insecticide for the Biological Control of Whiteflies in Greenhouses. Med. Fac. Landbouww. Univ. Gent. 60/3a:707-711.
- Fargues, J. M. Rougier, R. Goujet, et al. 1997. Inactivation of Conidia of *Paecilomyces fumosoroseus* by Near-Ultraviolet (UVB and UVA) and Visible Radiation. J. Invertebrate Pathology 69(1):70-78.
- Gerger, R. 2004. Characterisation of the Microbial Pest Control Agent *Paecilomyces fumosoroseus* strain FE 9901. Department de Microbiologia Universitat de Barcelona. Doc J-2c. Submitted to Futureco S.L. 5-12-2004.
- McNab, B.K. 1966. An Analysis of the Body Temperature of Birds. The Condor 68:47-55.



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## DATA EVALUATION RECORD

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**Reviewer:** Gail Tomimatsu, Ph.D. *gt*  
**Secondary Reviewer:** Shannon Borges, Team Leader *SB*

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**AUG 07 2014**

**STUDY TYPE:** Freshwater Fish Testing, Tier I (OCSPP 885.4200)

**MRID NO:** 49118303

**DP BARCODE:** 417625; 417629

**DECISION NO:** 481554; 481555

**SUBMISSION NO:** 938912; 938913

**TEST MATERIAL:** *Paecilomyces fumosoroseus* FE9901 (TGAI)

**STUDY NO:** 15706-11

**SPONSOR:** Novozymes BioAg, Inc., 13100 W. Lisbon Road, Suite 600, Brookfield, WI 53005

**TESTING FACILITY:** Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478

**TITLE OF REPORT:** *Paecilomyces fumosoroseus* FE9901. Final Report. Microbial Pest Control Agent (MPCA) Freshwater Fish Test with *Oncorhynchus mykiss* (Rainbow Trout)

**AUTHOR:** Mikulas, J.

**STUDY COMPLETED:** January 30, 2012

**CONFIDENTIALITY CLAIMS:** None.

**GOOD LABORATORY PRACTICE:** A signed and dated GLP statement was provided. The study was conducted in compliance with 40 CFR Part 160, with the following exceptions: 1) the provided certificate of analysis was not accompanied by a GLP compliance statement, and 2) mixture analysis was not performed.

**STUDY SUMMARY** A 30-day static-renewal laboratory bioassay was conducted to determine the toxicity of *Paecilomyces fumosoroseus* FE9901 to juvenile rainbow trout (*Oncorhynchus mykiss*). The fish were exposed to a concentration of  $1.0 \times 10^6$  cfu/mL as a suspension in synthetic freshwater, as well as by feed supplemented with the test material at a rate of at least  $1 \times 10^5$  cfu/mL. The test also included a sterile filtrate group exposed to a

suspension concentration equivalent to that of the test material group, and an untreated control group. Mortality in the test material group was 33.3%, significantly greater ( $p \leq 0.05$ ) than in the sterile filtrate group (0%) and the untreated control group (0%). The test solutions of the test material group appeared cloudy and/or the test chamber had a film from Day 15 until test end. The  $LC_{50}$  for *Paecilomyces fumosoroseus* FE9901 was determined greater than  $1 \times 10^6$  cfu/mL.

#### **CLASSIFICATION:**

**Supplemental.** Nominal doses of the test solutions were not measured or confirmed for viability in the testing laboratory, and test solutions were not confirmed for viability (i.e., dosage concentration), as recommended in OCSPP 885.4200. Additional details are provided in the EPA Reviewer's Comments and Conclusions.

#### **Test Material**

*Paecilomyces fumosoroseus* FE9901, Lot No. 100211, an off white-grey powder supplied by Natural Industries, Inc., Houston, TX with a reported potency of  $8.3 \times 10^9$  cfu/g. The manufacture date was given as 10-02-2011, and the expiration date as 01-01-2012. A certificate of analysis is provided on p. 18 of 19 of MRID 49118303. The Certificate of Analysis must be clarified and revised for the record. After receipt at the test facility on October 11, 2011, the test material was stored under refrigeration.

*Paecilomyces fumosoroseus* FE9901 sterile filtrate, Lot No. 111114, a brown liquid with a reported pre-filtration potency of  $2.43 \times 10^9$  cfu/g and a post-filtration potency of 0.00 cfu/g. A certificate of analysis is provided on p. 19 of 19 of MRID 49118303. The Certificate of Analysis must be clarified and revised for the record. After receipt at the test facility on November 17, 2011, the sterile filtrate was stored under refrigeration.

#### **Test Methods**

A 30-day static-renewal laboratory bioassay was conducted to determine the chronic toxicity and pathogenicity of the test material to juvenile rainbow trout (*Oncorhynchus mykiss*). The test fish were obtained from Trout Lodge, natality date unknown, but were assumed to be juvenile based on their size. The fish were held in the laboratory for at least 12 days, and were held in water of the same quality used in the test for at least seven days immediately prior to test start. No signs of disease, injury, stress, or mortality were seen during the acclimation period. At dosing, the fish weighed 0.5072 to 0.7822 g and were 29 to 33 mm long. The maximum loading rate was 1.0 g fish/L.

The test included a test material group exposed to a concentration of  $1.0 \times 10^6$  cfu/mL, a sterile filtrate group administered a concentration equivalent to that of the test material group, and an untreated control group. Each group contained three replicates of 10 fish each. In addition to the exposure via the test solution, the test material group fish received commercial fish food (Finfish Starter #1, ABS, Inc.) supplemented with the test material at a rate of ~0.035 g of test material per ~100 g of food. Prepared food was assigned an expiration day of seven days after preparation. The remaining fish groups received unaltered Finfish Starter #1. All fish were fed twice daily. Observations for survival, symptomatology, and abnormal behavior/appearance were made daily.

The test medium was moderately hard synthetic freshwater with a total hardness of 75 – 95 mg/L CaCO<sub>3</sub> and a pH of 7.8 – 8.2. The test material was administered as a suspension in the test medium. Test solutions for the group treated with the test material were prepared individually for each replicate by mixing 1.0 g of the test material into the appropriate volume of test medium to achieve a concentration of  $1 \times 10^6$  cfu/mL. The solution for the sterile filtrate group were prepared by mixing 3.0 mL of the sterile filtrate into the appropriate volume of test medium. Control group fish were exposed to untreated test medium only.

The test chambers were two-gallon glass aquaria filled with 8 L of the appropriate solution, producing a solution depth of ~22.5 cm. All aquaria were aerated throughout the test. The test chambers were maintained in an environmental chamber set to 12±2°C and a 16 hrs light/8 hrs darkness cycle.

The test solutions were prepared for renewal daily. At each renewal, approximately 80% of the old solution was siphoned from each test chamber and replaced with new test solution. During the renewal, the fish remained in the test chamber with approximately 20% of the old solution. Dissolved oxygen, temperature, conductivity, and pH of each replicate were measured in the new solutions on Days 1-27 and 29 and in the old solutions on Days 1-27, 29, and 30. The in-life phase of the testing was conducted Nov. 10, 2011 through Dec. 18, 2011.

The LC<sub>50</sub> was to be determined using ToxCalc v. 5.0.

### **Results Summary**

The following protocol deviations occurred: 1) the natality of the trout was unknown; 2) the fish in the test material group were fed expired food on Days 8-11; 3) physical parameters for the new solutions were not recorded on Days 0 and 28; 4) physical parameters for the old solutions were not recorded on Day 28; and 5) the pH was out of range in the old solutions on Day 21 and in the new solutions for the control and sterile filtrate groups on Day 24. These deviations did not adversely affect the study outcome.

During the test, the dissolved oxygen content ranged from 91% to 106% of saturation in the new solutions, and from 80% to 109% of saturation in the old solutions. The pH ranged from 7.4 to 8.2 in the new solutions and from 7.5 to 8.2 in the old solutions. Conductivity ranged from 297 to 396 µohms/cm in the new solutions, and from 279 to 476 µohms/cm in the old solutions. The temperature was 14°C in the new solutions and 13 to 14°C (taken from the chamber thermometer) in the old solutions.

After 30 days of exposure, cumulative mortality in the test material group was 33.3%, significantly greater ( $p \leq 0.05$ ) than in the sterile filtrate group (0%) and the untreated control group (0%). The test solutions of the test material group appeared cloudy and/or the test chamber had a film from Day 15 until test end. Fish in one replicate tank receiving the microbial treatment showed adverse effects 9 days after test initiation; and by day 13, there was at least 1 dead fish in each of 3 tanks (total = 5 dead). Since no symptomology was observed, there were no pathogenic effects observed. The LC<sub>50</sub> for *Paecilomyces fumosoroseus* FE9901 was greater than  $1 \times 10^6$  cfu/mL.

TABLE 1. Survival (%) of rainbow trout exposed to <i>Paecilomyces fumosoroseus</i> FE 9901																
	Study day															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Replicate																
Control A	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Control B	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Control C	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
<i>Paecilomyces</i> A	10	10	10	10	10	10	10	10	10	10	10	10	9	9	8	8
<i>Paecilomyces</i> B	10	10	10	10	10	10	10	10	10	10	10	10	9	9	9	8
<i>Paecilomyces</i> C	10	10	10	10	10	10	10	10	9	8	7	7	7	7	7	7
Sterile filtrate A	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Sterile filtrate B	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Sterile filtrate C	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Study day															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Survival (%)	
Control A	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	
Control B	10	10	10	10	10	10	10	10	10	10	10	10	10	10		
Control C	10	10	10	10	10	10	10	10	10	10	10	10	10	10		
<i>Paecilomyces</i> A	8	8	8	8	7	7	6	6	6	6	6	6	6	6	66.7	
<i>Paecilomyces</i> B	8	7	7	7	7	7	7	7	7	7	7	7	7	7		
<i>Paecilomyces</i> C	7	7	7	7	7	7	7	7	7	7	7	7	7	7		
Sterile filtrate A	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	
Sterile filtrate B	10	10	10	10	10	10	10	10	10	10	10	10	10	10		
Sterile filtrate C	10	10	10	10	10	10	10	10	10	10	10	10	10	10		

Data from pp. 10-11 of 19, MRID 49118303

### **Study Author's Conclusions**

The test was considered valid since control mortality was <30%. The study author concluded that the mean survival rate for the test material group was significantly lower than for the sterile filtrate and untreated control groups, indicating some toxic effects. The LC<sub>50</sub> for rainbow trout exposed to *Paecilomyces fumosoroseus* FE9901 in this study was >1 x 10<sup>6</sup> cfu/mL. Since no symptomatology was observed, there were no pathogenic effects observed.

### **EPA Reviewer's Conclusion and Comments:**

The reviewer agrees with the study author's conclusion that the mean survival rate for the test material group was significantly lower than for the sterile filtrate and untreated control groups, indicating possible microbial treatment-related toxicity or infectivity. Film or cloudy appearance in test chambers receiving solutions of the test material may have been a result of contaminated test solutions, or part of the fungal growth remaining in "old solutions". The presence of the cloudiness or film did not appear to adversely affect dissolved oxygen, however. The reviewer disagrees with the Study Author's conclusion: "no symptomatology was observed, there were no pathogenic effects observed", as there were no reported observations of necrotic tissues from moribund fish and no methodology on determination of examination of adversely affected fish. There were no observations to determine *Paecilomyces fumosoroseus* FE9901 dissemination, replication or survival in fish tissues, organs or fluids, as recommended in OPPTS guideline 885.4200.

Since nominal doses of the test solutions were not measured or confirmed for viability in the testing laboratory, and test solutions were not confirmed for viability (i.e., dosage concentration),

as recommended in OCSPP 885.4200, the study is classified as Supplemental. In addition, no steps were taken to ensure that the initial concentration of the MPCA was maintained throughout the test, as detailed in OPPTS Guideline 885.0001, and there was no explanation or justification for the relatively high mortalities in the test groups exposed to “viable” *Isária fumosoroseus* FE9901, and the mortalities did not appear to be related to water quality or physical interactions with the test substance.

The Certificates of Analysis for the test material and sterile filtrate must be clarified and revised for the record. The word “potency” in the context of this registration application is conceptually incorrect; the units “cfu/g” indicates microorganism viability in the test substance, not potency. However, if consistent potency is obtained with these units relative to a “standard inoculum-response curve (or a dose-response curve), please include the SOP, and explain how “potency” is determined (e.g., insect bioassay) in your response; or cite the study volume that provides the procedure(s) that Novozymes uses to determine potency. The reviewer believes that on the basis of the present study and other submitted ecotoxicology information that “potency” should be changed to “viability”.

The Lot Numbers for the test material and the test material filtered for the sterile filtrate are different. There is no immediate assurance that the test substance from Lot No. 100211 is sufficiently consistent to that of the test substance used to produce the sterile filtrate (Lot No. 111114), since the manufacturing dates differ by over 1 month; and the sterile filtrate control may not be a valid control. OPPTS Microbial Pesticide Test Guidelines (OPPTS885.0001-Overview for Microbial Pest Control Agents) recommend that the lot of the substance tested should be the same throughout the duration of the study, and the test sample should be stored under conditions that maintain purity and stability. If it is not possible to use the same lot throughout the test, subsequent lots of the test substance shall be selected to be as nearly identical to the original lot as practical. Chemical or biological assays shall be performed to ensure composition identity and consistency. The registrant is directed to provide details on the methodology and preparation of the test substance, Lot No. 100211 and the “age” of the production culture before shipment to the test facility.

Describe how viability (for verification of test material used in study; listed as potency on the Certificate of Analysis) was determined. If viability (cfu per unit weight or volume) was determined by serial dilution spread plating on a commercially available medium, please include the name of the medium (e.g., acidified potato dextrose agar is commonly used for fungal enumeration) and its components, i.e., “recipe” (e.g., Difco commercial preparation, dilute HCl used to acidify the agar medium to pH of 4.0) used to confirm the test material’s viability (and population) on the respective Certificates of Analysis. If relevant, citation to an SOP (if provided previously) in a previously submitted study volume could save time. Include a short description of the microbiology methodology and how the microbial populations were determined/quantified for the test material. An alternative procedure for quantifying the number of (germinable) spores in a hemacytometer may have been used to certify the viability/potency of the test material and the sterile filtrate control used in this study.

Measures of potency and/or viability are necessary QA/QC procedures for the manufacturer and ensure that all test material(s) for guideline testing are sufficiently representative of the pesticidal product(s). These QA/QC procedures should be established before commercial production of a pesticide to ensure consistency in product composition, manufacturing, purity and stability.

Unacceptable or incomplete Certificate(s) of Analysis may invalidate results of a test study (40 CFR §160.17).

The Certificate of Analysis for the test material and sterile filtrate must be clarified and revised for the record. The word "potency" in the context of this registration application is conceptually incorrect; the units "cfu/g" indicate microorganism viability in the test substance, not potency. However, if consistent potency is obtained with these units relative to a standard inoculum-response curve for a dose-response curve, please include the SOP, and explain how "potency" is determined (e.g., insect bioassay) to your response, or cite the study volume that provides the procedure(s) that Newbury uses to determine potency. The reviewer believes that on the basis of the present study and other substantial toxicology information that "potency" should be changed to "viability".

The Lot Numbers for the test material and the test material filtrate for the sterile filtrate are different. There is no immediate assurance that the test substance from Lot No. 100211 is sufficiently consistent to that of the test substance used to produce the sterile filtrate (Lot No. 111147) since the manufacturing dates differ by over 1 month, and the sterile filtrate control may not be a valid control. OPPT2882.0001-0-overview for Microbial Test Control Agency recommended that the lot of the substance tested should be the same throughout the duration of the study, and the test sample should be stored under conditions that maintain purity and stability. It is not possible to use the same lot throughout the test. Independent lots of the test substance shall be selected to be as nearly identical to the original lot as practical. Chemical or biological assays shall be performed to ensure composition identity and consistency. The applicant is directed to provide details on the methodology and preparation of the test substance, Lot No. 100211 and the "age" of the production culture before shipment to the test facility.

Describe how viability (for verification of test material used in study; listed as potency on the Certificate of Analysis) was determined. If viability (cfu per unit weight or volume) was determined by serial dilution spread plating on a commercially available medium, please include the name of the medium (e.g., acidified potato dextrose agar is commonly used for fungal enumeration) and its composition, i.e., "recipe" (e.g., Difco commercial preparation, dilute HCl used to adjust the agar medium to pH of 4.0) used to confirm the test material's viability (and population) on the respective Certificate of Analysis. If relevant, citation to an SOP (if provided previously) in a previously submitted study volume could save time. Include a short description of the microbiology methodology and how the microbial populations were determined/quantified in the test material. An alternative procedure for quantifying the number of (viable) spores in a hemocytometer may have been used to verify the viability/potency of the test material and the sterile filtrate control used in this study.

Measures of purity and/or viability are necessary QA/QC procedures for the manufacturer and ensure that all test material(s) for guidelines testing are sufficiently representative of the product(s). These QA/QC procedures should be established before commercial production of a pesticide to ensure consistency in product composition, manufacturing, purity and stability.



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**DATA EVALUATION RECORD****EPA Primary Reviewer:** Gail Tomimatsu, Ph.D., *gt***AUG 07 2014****EPA Secondary Reviewer:** Shannon Borges, Team Leader *SB*

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**STUDY TYPE:** Freshwater Aquatic Invertebrate Testing, Tier I (OCSPP 885.4240)**MRID NO:** 49118304**DP BARCODE:** 417625; 417629**DECISION NO:** 481554; 481555**SUBMISSION NO:** 938912; 938913**TEST MATERIAL:** *Paecilomyces fumosoroseus* FE9901 (TGAI)**STUDY NO:** 15707-11**SPONSOR:** Novozymes BioAg, Inc., 13100 W. Lisbon Road, Suite 600, Brookfield, WI 53005**TESTING FACILITY:** Stillmeadow, Inc., 12852 Park One Drive, Sugar Land, TX 77478**TITLE OF REPORT:** *Paecilomyces fumosoroseus* FE9901. Final Report.  
Microbial Pest Control Agent (MPCA) Freshwater Aquatic Invertebrate Test with *Daphnia magna***AUTHOR:** Mikulas, J.**STUDY COMPLETED:** February 2, 2012**CONFIDENTIALITY****CLAIMS:** None.**GOOD LABORATORY PRACTICE:**

A signed and dated GLP statement was provided. The study was conducted in compliance with 40 CFR Part 160, with the following exceptions: 1) the provided certificate of analysis was not accompanied by a GLP compliance statement, and 2) mixture analysis was not performed.

**STUDY SUMMARY:**

A 21-day static-renewal laboratory bioassay was conducted to determine the chronic toxicity of *Paecilomyces fumosoroseus* FE9901 to the freshwater invertebrate *Daphnia magna*. Groups of 50 neonate daphnids were exposed to a single limit dose of nominal concentration of  $1.0 \times 10^6$  cfu *Paecilomyces fumosoroseus* FE9901/mL in Elendt Medium. The test also included a sterile filtrate group and an untreated control group. There were no statistically significant differences among groups for

mortality (immobility) or mean dry weight of surviving adults. The mean number of neonates produced per surviving adult was significantly lower ( $p \leq 0.05$ ) in the test material group compared to the sterile filtrate and untreated control groups. The  $EC_{50}$  for mobility, reproduction, and growth was  $1.0 \times 10^6$  cfu *Paecilomyces fumosoroseus* FE9901/mL.

**CLASSIFICATION:**

**Supplemental.** Nominal doses of the test solutions were not measured or confirmed for viability in the testing laboratory, and test solutions were not confirmed for viability (i.e. cfu *Paecilomyces fumosoroseus* FE9901/mL), as recommended in OCSPP 885.4240. Additional details are provided in the EPA Reviewer's Comments and Conclusions.

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**Test Material**

*Paecilomyces fumosoroseus* FE9901, Lot No. 100211, an off white-grey powder supplied by Natural Industries, Inc., Houston, TX with a reported potency of  $8.3 \times 10^9$  cfu/g. The manufacture date was given as 10-02-2011, and the expiration date as 01-01-2012. A certificate of analysis is provided on p. 28 of 29 of MRID 49118304. The Certificate of Analysis must be clarified and revised for the record. After receipt at the test facility on October 11, 2011, the test material was stored under refrigeration.

*Paecilomyces fumosoroseus* FE9901 sterile filtrate, Lot No. 111114, a brown liquid with a reported pre-filtration potency of  $2.43 \times 10^9$  cfu/g and a post-filtration potency of 0.00 cfu/g. A certificate of analysis is provided on p. 29 of 29 of MRID 49118304. After receipt at the test facility on November 17, 2011, the sterile filtrate was stored under refrigeration. The Certificate of Analysis must be clarified and revised for the record. The Certificate of Analysis (CofA), on p. 29 is incorrectly titled and the Lot No tested may be different than reported. The submitted CofA is entitled: Microbial Pest Control Agent (MPCA) Freshwater Fish Test with *Oncorhynchus mykiss* (Rainbow Trout)". A corrected CofA with the Lot Number verified must be submitted for the record.

**Test Methods**

A 21-day static-renewal laboratory bioassay was conducted to determine the chronic toxicity of the test material to the freshwater invertebrate *Daphnia magna*. Neonate (<24 hrs old) daphnids from the test facility in-house culture were used in the test. Prior to dosing, the brood daphnids were held for at least 48 hours in water of the quality used in the test.

The test included a test material group exposed at a concentration of  $1.0 \times 10^6$  cfu/mL, a sterile filtrate group administered an amount equivalent to that of the test material group, and an untreated control group in test medium only. Each group contained 25 replicates of 2 daphnids each. During the test, the daphnids were fed daily with *S. capricornutum* and YCT.

The test medium was Elendt Medium M4, with a total hardness of 190 – 260 mg/L as CaCO<sub>3</sub> and a pH of 7.8 – 8.4. Test solutions for the test material group were prepared by mixing 0.5 g of the test material with 100 mL of deionized water. This stock solution was then mixed into the appropriate volume of test medium. Test solutions for the sterile filtrate group were prepared by mixing 0.5 mL of the sterile filtrate into the appropriate volume of test medium. The test solutions were prepared for renewal daily. At each renewal, the daphnids were pipetted from the old test solutions into the new test solutions. Dissolved oxygen, temperature, conductivity, and pH were measured daily in the new and old test solutions.

The test containers were 250 mL glass beakers containing 150 mL of the appropriate solution to produce a water depth of ~5 cm. The test containers were maintained in an environmentally controlled chamber set to 20±1°C with a 16 hrs light/8 hrs dark cycle.

The daphnids were examined daily for immobility, abnormal behavior/appearance, number of live offspring, and surviving adults. At test end the dry weight of each survivor was determined.

The EC<sub>50</sub> was to be determined using ToxCalc v. 5.0.

### **Results Summary**

The following protocol deviations occurred: 1) the daphnids were fed only algae on Day 17; 2) the conductivity was not recorded on Day 1 in the old solutions for the test material group; 3) the temperature was out of range in the new test solutions for all groups on Days 2 and 3. These deviations did not have an adverse effect on the study outcome.

During the test, the dissolved oxygen content ranged from 82% to 98% of saturation in the new solutions, and from 28% to 100% of saturation in the old solutions. The pH ranged from 7.1 to 8.3 in the new solutions and from 7.1 to 7.9 in the old solutions. Conductivity ranged from 569 to 737 µohms/cm in the new solutions, and from 598 to 739 µohms/cm in the old solutions. The solution temperature was 21-22°C in the new solutions and 21°C (read from the chamber thermometer) in the old solutions.

Mortality (immobility) was 10% in the untreated control and the test material groups, and 6% in the sterile filtrate group (Table 1). The net weight per mobile adult in the test material group was 1.274 mg, compared to 1.051 mg in the sterile filtrate group and 1.104 mg in the untreated control group. The average number of neonates per surviving adult in the test material group was 212, compared to 318 in the sterile filtrate group and 312 in the untreated control group.

<b>TABLE 1. Mobility, biomass, and neonate production for <i>Daphnia magna</i> exposed to <i>Paecilomyces fumosoroseus</i> FE9901 for 21 days</b>						
<b>Treatment</b>	<b>No. mobile adults</b>	<b>Percent mobile adults</b>	<b>Net weight per mobile adult (mg)</b>	<b>Percent difference from control</b>	<b>Total no. of neonates</b>	<b>Avg. no. of neonates per surviving adult</b>
Untreated control	45	90	1.104	--	14,018	312
<i>Paecilomyces fumosoroseus</i> FE9901 1.0 x 10 <sup>6</sup> cfu/mL	45	90	1.274	15.4	9550	212 <sup>a</sup>
Sterile filtrate	47	94	1.051	-4.8	14,933	318

<sup>a</sup>Significantly different from untreated control and sterile filtrate groups (p≤0.05)

Data from p. 10 of 29, MRID 49118304

The EC<sub>50</sub> for mobility, reproduction, and growth was  $>1.0 \times 10^6$  cfu/mL.

### **Study Author's Conclusions**

The study author concluded that the EC<sub>50</sub> for mobility, reproduction, and growth was  $>1.0 \times 10^6$  cfu/mL. Mean mobility and growth rates among the groups were not significantly different; however, the mean reproduction rate in the test material group was significantly lower compared to the remaining two groups.

### **EPA Reviewer's Conclusion and Comments:**

The reviewer agrees with the study author's conclusions, except for the conclusion "the mean reproduction rate in the test material group was significantly lower compared to the remaining two groups." Rate implies a parameter that is measured "over time" or "over distance". The mean number of offspring/surviving adult (neonates), and the mean number of neonates in the group exposed to  $1 \times 10^6$  *P. fumosoroseus* FE9901 cfu/mL was significantly reduced from groups receiving no treatment or that exposed to the sterile filtrate control. In this study, the NOEC for daphnid reproduction (fertility) and/or fecundity was  $< 1 \times 10^6$  *P. fumosoroseus* FE9901 cfu/mL.

Since nominal doses of the test solutions were not measured or confirmed for viability in the testing laboratory, and test solutions were not confirmed for viability (i.e., dosage concentration), as recommended in OCSPP 885.4240, there is uncertainty in the tested materials because verification of viability was not reported, and the study is classified as Supplemental. In addition, no steps were taken to ensure that the initial concentration of the MPCA was maintained throughout the test, as detailed in OPPTS Guideline 885.0001. The study is supplemental because a definitive endpoint was not defined for reproduction. Since adverse effects were observed at a presumed limit dose, they should have tested at lower concentrations to determine at least a NOEC, if not an EC<sub>50</sub> for reproductive effects.

The Certificates of Analysis for the test material and sterile filtrate must be clarified and revised for the record. The word "potency" in the context of this registration application is conceptually incorrect; the units "cfu/g" indicates microorganism viability in the test substance, not potency. However, if consistent potency is obtained with these units relative to a "standard inoculum-response curve (or a dose-response curve), please include the SOP, and explain how "potency" is determined (e.g., insect bioassay) in your response; or cite the study volume that provides the procedure(s) that Novozymes uses to determine potency. The reviewer believes that on the basis of the present study and other submitted ecotoxicology information that "potency" should be changed to "viability".

The Lot Numbers for the test material and the test material filtered for the sterile filtrate are different. There is no immediate assurance that the test substance from Lot No. 100211 is sufficiently consistent to that of the test substance used to produce the sterile filtrate (Lot No. 111114), since the manufacturing dates differ by over 1 month; and the sterile filtrate control may not be a valid control. OPPTS Microbial Pesticide Test Guidelines (OPPTS885.0001-Overview for Microbial Pest Control Agents) recommend that the lot of the substance tested should be the same throughout the duration of the study, and the test sample should be stored under conditions



that maintain purity and stability. If it is not possible to use the same lot throughout the test, subsequent lots of the test substance shall be selected to be as nearly identical to the original lot as practical. Chemical or biological assays shall be performed to ensure composition identity and consistency. The registrant is directed to provide details on the methodology and preparation of the test substance, Lot No. 100211 and the "age" of the production culture before shipment to the test facility.

Describe how viability (for verification of test material used in study; listed as potency on the Certificate of Analysis) was determined. If viability (cfu per unit weight or volume) was determined by serial dilution spread plating on a commercially available medium, please include the name of the medium (e.g., acidified potato dextrose agar is commonly used for fungal enumeration) and its components, i.e., "recipe" (e.g., Difco commercial preparation, dilute HCl used to acidify the agar medium to pH of 4.0) used to confirm the test material's viability (and population) on the respective Certificates of Analysis. If relevant, citation to an SOP (if provided previously) in a previously submitted study volume could save time. Include a short description of the microbiology methodology and how the microbial populations were determined/quantified for the test material. An alternative procedure for quantifying the number of (germinable) spores in a hemacytometer may have been used to certify the viability/potency of the test material and the sterile filtrate control used in this study.

Measures of potency and/or viability are necessary QA/QC procedures for the manufacturer and ensure that all test material(s) for guideline testing are sufficiently representative of the pesticidal product(s). These QA/QC procedures should be established before commercial production of a pesticide to ensure consistency in product composition, manufacturing, purity and stability. Unacceptable or incomplete Certificate(s) of Analysis may invalidate results of a test study (40 CFR §160.17).



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**DATA EVALUATION RECORD****AUG 07 2014****Reviewer:** Gail Tomimatsu, Ph.D.   
**Secondary Reviewer:** Shannon Borges, Team Leader 

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**STUDY TYPE:** Honeybee Testing (Nonguideline)

**MRID NO:** 47970501

**DP BARCODE:** 417625; 417629

**DECISION NO:** 481554; 481555

**SUBMISSION NO:** 938912; 938913

**TEST MATERIAL:** FUTURECO NOFLY™ (a.i., 18% w/w *Paecilomyces fumosoroseus* strain FE 9901)

**STUDY NO:** SRS06-SP01-82IR

**SPONSOR:** Natural Industries, Inc., 6223 Theall Rd., Houston, TX 77066

**TESTING FACILITY:** Recerca Agricola/SynTech Research Spain S.L., Pol. Ind. Norte, C-6, P-33-C, E-46230 (Alginet) Valencia, Spain

**TITLE OF REPORT:** Laboratory Trials to Test the Side Effect of NoFly WP and Adults of *Apis mellifera*: One Acute Oral and One Contact Toxicity Trial

**AUTHORS:** Corts, V., and S. Aucejo

**STUDY COMPLETED:** July 12, 2007

**CONFIDENTIALITY CLAIMS:** None.

**GOOD LABORATORY PRACTICE:** A signed and dated GLP statement was provided. The study meets the requirements of 40 CFR Part 160.

**STUDY SUMMARY:** An "outdoor, semi-contained" test was conducted to evaluate the effects of the test material to adult honeybees (*Apis mellifera* sp. *iberiensis*) when administered once in sucrose solution diet (sugar:water, 1:1) or topically at a nominal rate equivalent to the highest product label application rate (400 g/hL). The test also included a sucrose solution-only diet control, and a reference control, Dursban 75 WG (75% w/w chlorpyrifos). Bees in the diet trial were fed treated sucrose solution for approximately six hours, then untreated sucrose solution until test end. Bees in the topical trial were sprayed for two seconds using a uniform drop dispenser and fed untreated sucrose solution. The bees were observed for mortality

until all bees died by Day 11. By Day 8 of the diet trial, mortality was 60% in the test material group and 47.5% in the untreated control group. By Day 13 of the topical trial, mortality was 70% in the test material group and 57.5% in the untreated control group. Due to the high control mortality, results for only the first seven days of the diet trial and only the first 10 days of the topical trial were used to assess toxicity of the test material. According to the proposed OILB (Organisation Internationale de Lutte Biologique et Intégrée) classification scheme, the test material would be classified as “slightly toxic” (although the study authors stated it was not toxic) when administered in the diet and as “not toxic” when administered topically.

#### **CLASSIFICATION:**

**Unacceptable.** The results are inconclusive for determining potential hazards posed by exposures to viable *Paecilomyces fumosoroseus* FE9901. In addition, the study lacks confirming information regarding the tested materials, and verification of test/dietary solutions. Details regarding these deficiencies are provided in the EPA Reviewers’ Comments and Conclusions. Precautionary language protective for foraging bees is recommended.

#### **Test Material**

Futureco NoFly WP (a.i., 18% w/w *Paecilomyces fumosoroseus* strain FE 9901), Batch No. NF 060505, supplied by the study sponsor with an expiration date of November 9, 2006. After receipt at the test facility on May 11, 2006, the test material was stored under refrigeration at 4°C. Page 14 of 49 states that a certificate of analysis was received with the test material. A Certificate of Authenticity (or Analysis of the reported test material) was not included in MRID 47970501.

#### **Test Methods**

A laboratory test was conducted to evaluate the effects of the test material to adult honeybees (*Apis mellifera* sp. *iberiensis*) when administered once in sucrose solution diet (sugar:water, 1:1) or topically at a nominal rate equivalent to the highest product label application rate (400 g/hL; i.e., 400 g/100 L). The test also included a sucrose solution-only control, and a reference control, Dursban 75 WG (75% w/w chlorpyrifos).

The test bees were obtained from a single commercial beehive in Carcaixent (Valencia), and were selected with the help of a beekeeper to be representative of a normal population and to be food-seekers. Bees in the feeding trial were put into 0.5 L plastic cages; those in the topical trial were put in wooden cages (14 x 15 cm<sup>2</sup>). Each cage contained 10 bees and the cages were replicated four times (40 bees/group) and included in a randomized complete block design in an outside environment. Daily temperature and relative humidity data were recorded by a data logger at the field location.

For bees in the feeding trial, 200 g of water and 200 g of sugar were mixed with the appropriate amount of test material. A known amount of solution (Table 1) was taken from the mixing container with a 10 mL syringe (one per replicate) and the syringe was weighed. The tip of the syringe was covered with a small piece of yellow absorbent cloth to allow the bees to feed, and was inserted into the cage. After about 6 hours, the syringe was removed and reweighed. The bees were then supplied with plain sucrose solution that was replaced daily until test end.

For bees in the topical trial, a calibrated uniform drop dispenser containing 100 g of water, 100 g of sugar and the appropriate amount of test material was weighed and coupled to compressed air propellant. The dispenser was inserted into the cage and a 2-second application (~ 2 g) was made so that all bees received the product (Table 2). The dispenser was then reweighed, and a 5 mL syringe of sucrose solution was inserted into the top of the cage for feeding until test end.

<b>TABLE 1. Treatment for honeybees administered NoFly WP via feeding solution</b>	
Actual solution weight in syringe (g) <sup>a</sup>	1.98
Rate a.i. (g/hL)	72
Actual f.p. in syringe (g) <sup>a</sup>	$3.94 \times 10^{-3}$
Actual f.p. taken by 10 bees (g) <sup>a</sup>	$3.46 \times 10^{-4}$
Actual f.p. taken by 1 bee (g) <sup>a</sup>	$3.46 \times 10^{-5}$
Amount of a.i./bee (g) <sup>a</sup>	$6.23 \times 10^{-6}$

<sup>a</sup>Average from 3 replicates

Data from p. 15 of 49, MRID 47970501

f.p. was not defined

<b>TABLE 2. Treatment for honeybees administered NoFly WP via topical spray</b>	
Spraying time (sec)	2
Actual solution sprayed (g) <sup>a</sup>	2.13
Rate a.i. (g/hL)	72
Actual f.p. taken by 10 bees (g) <sup>a</sup>	$4.24 \times 10^{-3}$
Actual f.p. applied to 1 bee (g) <sup>a</sup>	$4.24 \times 10^{-4}$
Amount of a.i./bee (g) <sup>a</sup>	$7.63 \times 10^{-5}$

<sup>a</sup>Average from 4 replicate cages, each replicate cage had 10 bees

Data from p. 16 of 49, MRID 47970501

f.p. was not defined

Beginning 24 hours after application, the bees were observed daily (with a few exceptions) and the number of dead bees was recorded. Observations continued until all the bees had died by Day 11.

To determine if there was a significant difference in mortality among the test groups, the data were analyzed using ANOVA followed by Dunnett's test, using the commercial program ARM 7.3.6. Those results were verified using the commercial program Statgraphics 5.0. Mortality in the test material and reference control groups was corrected for control mortality using the Schneider-Orelli modification to Abbott's formula.

## **Results Summary**

Results for the feeding trial are summarized in Table 1. The trial was considered valid, since untreated control mortality was below 15% during the first 72 hours. This was stated to be in the estimated time in which the test material affects the target pest (whiteflies). Mortality in the reference control was 100% at 24 hours. By Day 8, mortality in the test material group was 60%, but was also high (47.5%) in the untreated control group, leading the study authors to suggest that

the 60% mortality rate could not be attributed solely to the test material. Therefore, results for the only the first seven days post-treatment were used to assess toxicity of the test material. The study authors stated that mortality in the test material group during the first seven days was below 25%, classifying the test material as not toxic according to the proposed OILB classification scheme:

1 = not toxic (<25%)

2 = slightly toxic (25-50%)

3 = medium toxic (51-75%)

4 = toxic (>75%).

<b>TABLE 1. Mortality of honeybees treated with Futureco NoFly via diet</b>									
<b>Treatment</b>	<b>Time after treatment</b>								
	<b>24 hr</b>	<b>48 hr</b>	<b>72 hr</b>	<b>4 days</b>	<b>7 days</b>	<b>8 days</b>	<b>9 days</b>	<b>10 days</b>	<b>11 days</b>
<b>Average number of dead</b>									
Untreated control	0b	0b	0b	0.25b	3.0b	4.75a	7a	7.5a	10
Futureco NoFly	0b	0b	0b	0b	0.33c	6a	8.3a	10a	10
Dursban 75	10a	10a	10a	10a	10a	10a	10a	10a	10
<b>Percent mortality</b>									
Untreated control	0a	0a	0a	2.5a <sup>1</sup>	30a	47.5a	70a	75a	100
Futureco NoFly	0a	0a	0a	0a	3.3a	60a	83.3a	100a	100
Dursban 75	100b	100b	100b	100b	100b	100a	100a	100a	100
<b>Corrected mortality (%)<sup>a</sup></b>									
Futureco NoFly	0a	0a	0a	0a	0a	23.81b	44.33b	100b	--
Dursban 75	100b	100b	100b	100b	100b	100b	100b	100b	--
<b>Toxicity class<sup>b</sup></b>									
Futureco NoFly	1	1	1	1	1	1	--	--	--
Dursban 75	4	4	4	4	4	4	--	--	--

Data from p. 15 of 46, 17-18 of 46, MRID 47970501

<sup>a</sup>Corrected for control mortality using the formula  $((\%MP - \%MC) / (100 - \%MC)) * 100$ , where %MP = mortality in the test material group, %MC = mortality in the untreated control group

<sup>b</sup>According to OILB criteria, where 1 = no toxicity (<25%), 2 = slight toxicity (25-50%), 3 = medium toxicity (51-75%), 4 = toxic (>75%)

Means followed by the same letter do not differ

<sup>1</sup>Given as 0.0 in Table 5, p. 17 of 46, MRID 47970501

Results for the contact trial are summarized in Table 2. The trial was considered valid, since untreated control mortality was below 15% during the first 72 hours. Mortality in the reference control was 100% at 24 hours. By Day 13, mortality in the test material group was 70%, but was also high (57.5%) in the untreated control group, leading the study authors to suggest that the 70% mortality rate could not be attributed solely to the test material. Therefore, results for the first 10 days post-treatment were used to assess toxicity of the test material. Mortality during the first seven days was below 25%, classifying the test material as not toxic according to the proposed OILB classification scheme.

<b>TABLE 1. Mortality of honeybees treated with Futureco NoFly via contact</b>												
Treatment	Time after treatment											
	24 hr	48 hr	72 hr	6 days	7 days	8 days	9 days	10 days	13 days	14 days	15 days	16 days
Average number of dead												
Untreated control	0b	0b	0b	0b <sup>1</sup>	0.25c	0.25c	0.25c	0.50c	5.75a	7.75a	8a	10a
Futureco NoFly	0b	0b	0b	0.67b	1b	1.33b	1.67b	3.33b	7a	8.33a	10a	10a
Dursban 75	10a	10a	10a	10a	10a	10a	10a	10a	10a	10a	10a	10a
Percent mortality												
Untreated control	0a	0a	0a	0a <sup>2</sup>	2.5a	2.5a	2.5a	5a	57.5a	77.5a	80a	100a
Futureco NoFly	0a	0a	0a	6.67a	10b	13.33b	16.67b	33.3b	70.00a	83.33a	100a	100a
Dursban 75	100b	100b	100b	100b	100b	100b	100b	100b	100a	100a	100a	100a
Corrected mortality (%) <sup>a</sup>												
Futureco NoFly	0a	0a	0a	6.67b <sup>3</sup>	7.69b	11.11b	14.3b	29.82b	29.41b	25.93b	100b	--
Dursban 75	100b	100b	100b	100b	100b	100b	100b	100b	100b	100b	100b	--
Toxicity class <sup>b</sup>												
Futureco NoFly	1	1	1	1	1	1	1	2				
Dursban 75	4	4	4	4	4	4	4	4				

Data from p. 20 of 46, 22-24 of 46, MRID 47970501

<sup>a</sup>Corrected for control mortality using the formula  $((\%MP - \%MC) / (100 - \%MC)) * 100$ , where %MP = mortality in the test material group, %MC = mortality in the untreated control group

<sup>b</sup>According to OILB criteria, where 1 = no toxicity (<25%), 2 = slight toxicity (25-50%), 3 = medium toxicity (51-75%), 4 = toxic (>75%)

Means followed by the same letter do not significantly differ

<sup>1</sup>Given as 0.25 in Table 10, p. 22 of 46, MRID 47970501

<sup>2</sup>Given as 0.25 in Table 10, p. 23 of 46, MRID 47970501

<sup>3</sup>Given as 4.3 in Table 10, p. 24 of 46, MRID 47970501

### **Study Authors' Conclusion**

The study authors concluded that, under the trial conditions, the test material administered either orally or topically was innocuous to honeybees during the three days immediately after application.

### **EPA Reviewer's Conclusions and Comments:**

Twenty percent bee mortality was observed in the untreated controls for the oral and contact exposures within 4 to 7 days for the oral study; and within 10 to 13 days for the contact study. Percent bee mortality in the untreated controls was not statistically different than that of the bees exposed to *Pf*FE9901 in the oral exposure test. Percent bee mortality in the untreated controls was statistically different than that of bees exposed to *Pf*FE9901 in the contact exposure test between 6 to 10 days after exposure. Bee mortalities may have been confounded by the test design and/or system, and the results are inconclusive for determining potential hazards posed by exposure to viable *Paecilomyces fumosoroseus* FE9901.

The study report indicated that three days is the estimated time in which the test material affects the target pests, and the product label states that it typically takes 3-7 days for an infected insect to die, and about 7 to 10 days after the first spray to see a reduction in an insect population.

Assuming that honeybees are similarly sensitive or susceptible to *Paecilomyces fumosoroseus* FE9901, the results reported in this study support targeted pest insect claims, however bee mortalities were also observed in the untreated controls.

The study states that a certificate of analysis was provided to the test facility, but it was not included in MRID 47970501.

This nonguideline study is classified as Unacceptable. Without an acceptable Certificate of Analysis, and a study which incorporates several procedural considerations (OPPTS 885.0001-Overview for Microbial Pest Control Agents (g)(1-3)) for verifying or confirming dosing or feeding solutions, the study is not useful for a regulatory risk assessment to characterize potential microbial pesticide hazards to honeybees.



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**DATA EVALUATION RECORD**

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EPA Primary Reviewer: Gail Tomimatsu, Ph.D., *gt***AUG 07 2014**EPA Secondary Reviewer: Shannon Borges, Team Leader *SB*

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**STUDY TYPE:** Nontarget Insect Testing, Tier I (OCSPP 885.4340)

**MRID NO:** 49118305

**DP BARCODE:** 417625; 417629

**DECISION NO:** 481554; 481555

**SUBMISSION NO:** 938912; 938913

**TEST MATERIAL:** FUTURECO NOFLY™ (a.i., 18% w/w *Paecilomyces fumosoroseus* strain FE 9901)

**STUDY NO:** Not provided

**SPONSOR:** Novozymes BioAg, Inc., 13100 W. Lisbon Road, Suite 600, Brookfield, WI 53005

**TESTING FACILITY:** Not provided

**TITLE OF REPORT:** Evaluation of Side Effects of NoFly WP to Whitefly Natural Enemies in the Canary Islands

**AUTHORS:** H., A.C.; A.P. Cubas; E.H. Suarez, et al.

**STUDY COMPLETED:** October 30, 2004

**CONFIDENTIALITY CLAIMS:** None.

**GOOD LABORATORY PRACTICE:** A signed and dated GLP statement was provided. The study does not meet the requirements of 40 CFR Part 160. No Quality Assurance unit was in place.

**STUDY SUMMARY:** Six laboratory tests and one semi-field test were conducted to determine the effects of Futureco NoFly (*Paecilomyces fumosoroseus* strain FE 9901) on mortality and reduction in parasitism (where applicable) of some parasitoids and predators of the whitefly (*Trialeurodes vaporariorum*). The tests also included a reference control and untreated control group. In the laboratory tests, the insects were exposed by contact with leaf discs from plants sprayed at an application rate of  $1 \times 10^5$  cfu/cm<sup>2</sup> of leaf. The test material was classified as innocuous to adults of the parasitoids *Encarsia formosa* and *Eretmocerus mundus*. Futureco NoFly significantly increased mortality of first-stage nymphs of the predator *Macrolophus caliginosus* and

was classified as slightly dangerous, requiring a second test with a less sensitive life stage. Abbott-corrected mortality in fourth-stage *M. caliginosus* nymphs was 94%, classifying the test material as toxic, but the high mortality was thought to be due to the test material growing on the insect food source (*Ephestia kuehniella* eggs) and enhancing exposure. Corrected mortality in first-stage nymphs of the predator *Orius laevigatus* was 100%, classifying the test material as toxic and requiring a trial with a less sensitive life stage. Corrected mortality of fourth-stage nymphs of *O. laevigatus* was 73%, classified as moderately dangerous and requiring a semi-field test. In the semi-field test, first stage nymphs of *O. laevigatus* were exposed on whole plants that had been sprayed with an application rate of  $5 \times 10^4$  cfu/cm<sup>2</sup> leaf. Corrected mortality was 23%, classifying the test material as innocuous.

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**CLASSIFICATION:** Supplemental

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### **Test Material**

Futureco NoFly WP (a.i., 18% w/w *Paecilomyces fumosoroseus* strain FE 9901), Batch No. 252003 BD; with a reported concentration of  $6.77 \times 10^8$  cfu/g in five studies and  $7.16 \times 10^8$  cfu in two studies. No further information was provided. No Certificate of Analysis was provided with the study report.

### **Test Methods**

Seven separate tests were conducted to determine the effects of the test material to four natural predators of whiteflies in the Canary Islands. The most susceptible life stage of the particular predator was used for the tests. The tests were based on the method of Hassan et al. (1994), which is included in the IOBC/OILB guidelines to evaluate side effects of plant protection products to nontarget arthropods (Candolfi et al., 2000). The endpoints used were mortality and reduction in parasitism. Mortality was corrected for control mortality using Abbott's formula:

$$\frac{\%MP - \%MC}{100 - \%MC} \times 100 \text{ where}$$

%MP = mortality in the treated group

%MC = mortality in the untreated control.

#### **Trial 1**

A laboratory study was conducted to determine the effects of contact exposure of the test material on mortality and reduction in parasitism in adults of the whitefly parasitoid *Encarsia formosa*. The

test also included a positive control group exposed to cypermethrin (1.5 cc/L sterile tap water) and an untreated control (sterile tap water only).

The test organisms were obtained from parasitized whitefly (*Trialeurodes vaporariorum*) pupae and were exposed to the appropriate treatment in individual containers. Twenty leaf discs (2.5 cm diameter) were taken from plants (not identified) treated by spraying to runoff with the test material ( $1 \times 10^5$  cfu/cm<sup>2</sup> leaf), the positive control (1.5 cc/L), or sterile water. Each leaf disc was placed on a 2.5 cm diameter agar disc and one *E. formosa* adult was placed on top. A sterile filter paper strip wetted with a 1:1 honey:water mixture was added to each container as a food source. Each treatment group of 20 test organisms was replicated four times.

The test containers were maintained at  $25 \pm 2^\circ$  C and >80% humidity with a photoperiod of 16 hrs light:8 hrs darkness. Mortality was recorded 24 hours post-exposure.

To evaluate parasitism, the treated leaf discs were removed and replaced by freshly-treated discs containing *T. vaporariorum* nymphs. The discs were placed in Petri dishes containing wetted sterile sand. The discs were replaced daily and parasitism was recorded for six days. The percent reduction in parasitism was calculated as

$$\frac{R_c - R_t}{R_c} \times 100 \text{ where}$$

$R_t$  = parasitism with the test material treatment

$R_c$  = parasitism with the control treatment.

The test material is considered innocuous if parasitism is reduced by less than 50%, and toxic if it is reduced by more than 50% (Oomen, 1985).

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin  $\sqrt{x}$  function and analyzed using the Kruskal-Wallis test ( $p \leq 0.05$ ).

## Results Summary

Results are summarized in Table 1. There was no statistically significant difference in mortality or reduction in parasitism between the test material and untreated control group. Mortality and reduction in parasitism in the reference control was significantly higher than in the other two groups.

TABLE 1. Effect of Futureco NoFLy on mortality and parasitism of <i>Encarsia formosa</i> adults		
Treatment	Mortality (% $\pm$ SE)	Reduction of parasitism (% $\pm$ SE)
Untreated control	3.8 $\pm$ 2.39a	--
Futureco NoFly	0 $\pm$ 0a	11.4 $\pm$ 4.71b
Reference control	77.5 $\pm$ 3.73b	58.7 $\pm$ 1.88a

Data from p. 7, MRID 49118305

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

Corrected mortality is given in Table 2. The test material was classified as innocuous to *E. formosa* adults when applied at a rate of  $1 \times 10^5$  cfu/cm<sup>2</sup> leaf.

TABLE 2. Toxicity of Futureco NoFly to <i>Encarsia formosa</i> adults		
Parameter	Corrected mortality	Classification <sup>a</sup>
Corrected mortality	-30 ± 14.01	1
Reduction in parasitism	11.4 ± 4.71	1

Data from p. 7, MRID 49118305

<sup>a</sup>According to IOBC/WPRS classification (Hassan et al., 1994, 2000)

## Trial 2

A laboratory study was conducted to determine the effects of contact exposure of the test material on mortality and reduction in parasitism by adults of the whitefly parasitoid by *Eretmocerus mundus*. The test also included a positive control group exposed to cypermethrin (1.5 cc/L sterile tap water) and an untreated control (sterile tap water only).

The test organisms were obtained from parasitized whitefly (*Bemisia tabaci*) pupae and were exposed to the appropriate treatment in individual containers. Twenty leaf discs (2.5 cm diameter) were taken from tomato plants treated by spraying with the test material ( $1 \times 10^5$  cfu/cm<sup>2</sup> leaf), the positive control (1.5 cc/L), or sterile water. Each leaf disc was placed on a 2.5 cm diameter agar disc and one *E. mundus* adult was placed on top. A sterile filter paper strip wetted with a 1:1 honey:water mixture was added to each container as a food source. Each treatment group of 20 test organisms was replicated four times.

The test containers were maintained at  $25 \pm 2^\circ$  C and >80% humidity with a photoperiod of 16 hrs light:8 hrs darkness. Mortality was recorded 24 hours post-exposure.

To evaluate parasitism, the treated leaf discs were removed and replaced by freshly-treated cabbage leaf discs containing *Bemisia tabaci* nymphs. The discs were placed in Petri dishes containing wetted sterile sand. The discs were replaced daily and parasitism was recorded for six days. The percent reduction in parasitism was calculated as

$$\frac{R_c - R_t}{R_c} \times 100 \text{ where}$$

R<sub>t</sub> = parasitism with the test material treatment

R<sub>c</sub> = parasitism with the control treatment.

The test material is considered innocuous if parasitism is reduced by less than 50%, and toxic if it is reduced by more than 50% (Oomen, 1985).

The effect of mortality and parasitism together was evaluated using the following expression:

$$100\% - [(100\% - M) \times R] \text{ where}$$

M = MP/MC

R = R<sub>t</sub>/R<sub>c</sub> (mean values).

The risk classification of the latter evaluation was determined according to the toxicity categories of Hassan (1997) and Dusso et al., 1992):

Score	Denomination	Amended Mortality
1	Innocuous	<50%
2	Slightly dangerous	50-79%
3	Moderately dangerous	80-99%
4	Dangerous	>99%

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin  $\sqrt{x}$  function and analyzed using the Kruskal-Wallis test ( $p \leq 0.05$ ).

### Trial 2 Results

Results are summarized in Table 3. There was no statistically significant difference in mortality among the test material, untreated control, and reference material groups. Reduction in parasitism in the reference control was significantly higher than in the test material group.

<b>TABLE 3. Effect of Futureco NoFLy on mortality and parasitism of <i>Eretmocerus mundus</i> adults</b>		
Treatment	Mortality (% $\pm$ SE)	Reduction of parasitism (% $\pm$ SE)
Untreated control	11.3 $\pm$ 3.15	--
Futureco NoFly	16.3 $\pm$ 4.27	41.7 $\pm$ 6.09b
Reference control	12.5 $\pm$ 5.53	74.7 $\pm$ 4.38a

Data from p. 11, MRID 49118305

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

Corrected mortality is given in Table 4. The test material was classified as innocuous to *E. mundus* adults when applied at a rate of  $1 \times 10^5$  cfu/cm<sup>2</sup> leaf.

<b>TABLE 4. Toxicity of Futureco NoFLy to <i>Eretmocerus mundus</i> adults</b>		
Parameter	Result (%) ( $\pm$ SD)	Classification <sup>a</sup>
Corrected mortality	5.6 $\pm$ 3.87	1
Combined effect	42.7 $\pm$ 5.76	1
Reduction in parasitism	41.7 $\pm$ 6.09	1

Data from p. 11, MRID 49118305

<sup>a</sup>According to IOBC/WPRS classification (Hassan et al., 1977, 1994, 2000; Duso et al., 1992)

### Trial 3

A laboratory study was conducted to determine the effects of contact exposure of the test material on mortality of first-stage nymphs of the whitefly predator *Macrolophus caliginosus*. The test organisms were reared on bean pods at  $25 \pm 1^\circ\text{C}$  under 16 hrs light:8 hrs darkness. The test also included a toxic reference control group exposed to Deltamethrin 2.5 EC (0.04% in sterile tap water), a selective reference control group exposed to Atominal 10 EC (62.5 cm<sup>3</sup>/hL in sterile tap water), and an untreated control (sterile tap water only). The test material was applied at  $1 \times 10^5$  cfu/cm<sup>2</sup> leaf.

Leaf discs (2.5 cm diameter) were taken from four tomato plants (8-10 leaves) treated by spraying

the appropriate treatment to runoff. Each treatment consisted of three replicates of six discs/plant. Each leaf disc was placed in a ventilated Petri dish containing sand wetted with sterile water. A food source of *Ephestia kuehniella* eggs was placed on each leaf disc and one *M. caliginosus* nymph was placed in the dish.

The dishes were maintained at  $22 \pm 2^\circ\text{C}$  and 72% humidity with a photoperiod of 16 hrs light:8 hrs darkness. Eggs were provided on Days 1, 4, and every two or three days thereafter until test end. Mortality was recorded on Days 1, 2, 7, and 9 after test start. Missing nymphs were considered dead. The test is considered valid if control mortality is  $<25\%$ , and mortality in the standard reference control is  $>40\%$  (Bakker et al., 2000).

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin  $\sqrt{x}$  function and analyzed using the Kruskal-Wallis test ( $p \leq 0.05$ ).

### Trial 3 Results

Results are summarized in Table 5. Mortality in the test material group was significantly higher than in the untreated control on Day 7, and significantly higher than in any other group on Day 9.

Treatment	Day 1	Day 2	Day 7	Day 9
Untreated control	$0 \pm 0a$	$1.4 \pm 1.39a$	$8.3 \pm 2.41a$	$13.9 \pm 1.39a$
Futureco NoFly	$2.8 \pm 2.78a$	$4.2 \pm 4.17a$	$40.3 \pm 5.01b$	$75 \pm 2.41d$
Reference control	$22.2 \pm 1.39b$	$33.3 \pm 8.67b$	$44.4 \pm 7.35b$	$55.6 \pm 7.35b$
Selective reference control	$2.8 \pm 2.78a$	$6.9 \pm 2.78ab$	$11.1 \pm 2.78a$	$20.8 \pm 0c$

Data from p. 13, MRID 49118305

Different letters within a column indicate significant differences (Kruskal-Wallis,  $p \leq 0.05$ , days 1 and 9; ANOVA,  $p \leq 0.05$ , DSH Tukey,  $p \leq 0.05$  days 2 and 7)

Corrected mortality is given in Table 6. The toxicity of the test material was classified as 2 (slightly dangerous) to the first stage nymph of *M. caliginosus* seven and nine days after treatment. According to the IOBC guidelines, (Hassan et al, 1994; Bekker et al., 2000), a second laboratory test must be performed with a less sensitive stage.

Day	Corrected mortality (%)	Classification <sup>a</sup>
1	$2.8 \pm 2.78$	1
2	$2.9 \pm 2.9$	1
7	$34.5 \pm 7.06$	2
9	$70.9 \pm 3.24$	2

Data from p. 13, MRID 49118305

<sup>a</sup>According to IOBC/WPRS classification (Hassan et al., 1994, 2000)

### Trial 4

A laboratory study was conducted to determine the effects of contact exposure of the test material on mortality of fourth-stage nymphs of the whitefly predator *M. caliginosus*. The test organisms were reared on bean pods. The test also included a toxic reference control group exposed to

Deltamethrin 2.5 EC (0.04% in sterile tap water), a selective reference control group exposed to Atominal 10 EC (62.5 cm<sup>3</sup>/hL in sterile tap water), and an untreated control (sterile tap water only). The test material was applied at  $1 \times 10^5$  cfu/cm<sup>2</sup> leaf.

Leaf discs (2.5 cm diameter) were taken from four tomato plants (8-10 leaves) treated by spraying the appropriate treatment to runoff. Each treatment consisted of three replicates of six discs/plant. Each leaf disc was placed in a ventilated Petri dish containing sand wetted with sterile water. A food source of *Ephestia kuehniella* eggs was placed on each leaf disc and one *M. caliginosus* nymph was placed in the dish.

The dishes were maintained at  $22 \pm 2^\circ\text{C}$  and 82% humidity with a photoperiod of 16 hrs light:8 hrs darkness. Eggs were provided on Days 1, 2, 4, and every two days thereafter until test end. Mortality was recorded on Days 1, 2, 7, and 9 after test start. Missing nymphs were considered dead. The test is considered valid if control mortality is <25%, and mortality in the standard reference control is >40% (Bakker et al., 2000).

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin  $\sqrt{x}$  function and analyzed using the Kruskal-Wallis test ( $p \leq 0.05$ ).

#### Trial 4 Results

Results are summarized in Table 7. Mortality in the test material treatment was significantly higher than the other treatments on Day 9.

<b>Treatment</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 7</b>	<b>Day 9</b>
Untreated control	0 $\pm$ 0a	1.4 $\pm$ 1.39a	4.2 $\pm$ 2.41a	4.2 $\pm$ 2.41a
Futureco NoFly	0 $\pm$ 0a	0 $\pm$ 0a	87.5 $\pm$ 6.36b	94.4 $\pm$ 3.67b
Reference control	2.8 $\pm$ 2.78a	2.8 $\pm$ 2.78a	5.6 $\pm$ 2.78a	2 $\pm$ 1.33a
Selective reference control	0 $\pm$ 0a	0 $\pm$ 0a	1.4 $\pm$ 1.39a	4.2 $\pm$ 2.41a

Data from p. 15, MRID 49118305

Different letters within a column indicate significant differences ( $p \leq 0.05$ )

Corrected mortality is given in Table 8. The toxicity of the test material was classified as 3 (moderately dangerous) to the fourth stage nymph of *M. caliginosus* seven and nine days after treatment. According to the IOBC guidelines, (Hassan et al, 1994; Bekker et al., 2000), a third trial must be performed under semi-field conditions.

The study authors stated that the test material group mortality was much higher than expected, since stage four nymphs were expected to be less sensitive than stage one nymphs. This was believed to be due to the test material being able to develop and grow on the food source, exposing the stage four nymphs by repeated doses via the infected eggs, in addition to the direct contact exposure. On Day 7, mortality was higher in the test material group than in either of the reference control groups. The study authors stated that it appears that the method used does not suit the special characteristics of Futureco NoFly.

TABLE 8. Toxicity of Futureco NoFly to <i>M. caliginosus</i> fourth-stage nymphs		
Day	Corrected mortality (%)	Classification <sup>a</sup>
1	0 ± 0	1
2	-1.4 ± 1.45	1
7	86.6 ± 6.86	3
9	94.1 ± 3.82	3

Data from p. 15, MRID 49118305

<sup>a</sup>According to IOBC/WPRS classification (Hassan et al., 1994, 2000)

### Trial 5

A laboratory study was conducted to determine the effects of contact exposure of the test material on mortality of first-stage nymphs of the whitefly predator *Orius laevigatus*. The test organisms were reared on bean pods at 25±1°C under 16 hrs light:8 hrs darkness. The test also included a toxic reference control group exposed to Deltamethrin 2.5 EC (0.04% in sterile tap water), a selective reference control group exposed to Atominal 10 EC (62.5 cm<sup>3</sup>/hL in sterile tap water), and an untreated control (sterile tap water only). The test material was applied at 1 x 10<sup>5</sup> cfu/cm<sup>2</sup> leaf.

Leaf discs (2.5 cm diameter) were taken from four tomato plants (8-10 leaves) treated by spraying the appropriate treatment to runoff. Each treatment consisted of three replicates of six discs/plant. Each leaf disc was placed in a Petri dish containing sand wetted with sterile water. A food source of *Ephestia kuehniella* eggs was placed on each leaf disc and one *O. laevigatus* nymph was placed in the dish.

The dishes were maintained at 24 ±2°C and 76% humidity with a photoperiod of 16 hrs light:8 hrs darkness. Eggs were provided on Days 1, 4, and every two or three days thereafter until test end. Mortality was recorded on Days 1, 2, 7, and 9 after test start. Missing nymphs were considered dead. The test is considered valid if control mortality is <25%, and mortality in the standard reference control is around 40% (Bakker et al., 2000).

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin √x function and analyzed using the Kruskal-Wallis test (p≤ 0.05).

### Trial 5 Results

Results are summarized in Table 9. Mean mortality in the test material group was significantly higher than in the untreated control on Days 2, 7, and 9.

TABLE 9. Mean mortality (%) of <i>Orius laevigatus</i> first-stage nymphs exposed to Futureco NoFly				
Treatment	Day 1	Day 2	Day 7	Day 9
Untreated control	5.6 ± 2.78a	6.9 ± 1.39a	13.9 ± 1.39a	16.7 ± 4.17a
Futureco NoFly	5.6 ± 3.67a	16.7 ± 2.41c	98.6 ± 1.39b	100 ± 0b
Reference control	84.7 ± 7.73a	95.8 ± 4.17b	100 ± 0b	100 ± 0b
Selective reference control	1.4 ± 1.39a	9.37 ± 1.39ac	23.6 ± 5.01a	31.9 ± 8.45a

Data from p. 17, MRID 49118305

Different letters within a column indicate significant differences (p≤0.05)

Corrected mortality is given in Table 10. The toxicity of the test material was classified as 4 (dangerous) to the first stage nymph of *O. laevigatus* nine days after treatment, when mortality was 100%. According to the IOBC guidelines, (Hassan et al, 1994; Bekker et al., 2000), a new trial with a less sensitive stage must be performed.

<b>TABLE 10. Toxicity of Futureco NoFly to <i>O. laevigatus</i> first-stage nymphs</b>		
<b>Day</b>	<b>Corrected mortality (%)</b>	<b>Classification<sup>a</sup></b>
1	-0.1 ± 4.48	1
2	10.5 ± 1.59	1
7	98.4 ± 1.59	3
9	100 ± 0	4

Data from p. 17, MRID 49118305

<sup>a</sup>According to IOBC/WPRS classification (Hassan et al., 1994, 2000)

### Trial 6

A laboratory study was conducted to determine the effects of contact exposure of the test material on mortality of fourth-stage nymphs of the whitefly predator *Orius laevigatus*. The test organisms were reared on bean pods at 25±1°C under 16 hrs light:8 hrs darkness. The test also included a toxic reference control group exposed to Deltamethrin 2.5 EC (0.04% in sterile tap water), a selective reference control group exposed to Atominal 10 EC (62.5 cm<sup>3</sup>/hL in sterile tap water), and an untreated control (sterile tap water only). The test material was applied at 1 x 10<sup>5</sup> cfu/cm<sup>2</sup> leaf.

Leaf discs (2.5 cm diameter) were taken from four tomato plants (8-10 leaves) treated by spraying the appropriate treatment to runoff. Each treatment consisted of three replicates of six discs/plant. Each leaf disc was placed in a Petri dish containing sand wetted with sterile water. A food source of *Ephestia kuehniella* eggs was placed on each leaf disc and one *O. laevigatus* nymph was placed in the dish.

The dishes were maintained at 24 ±2°C and 72% humidity with a photoperiod of 16 hrs light:8 hrs darkness. Eggs were provided on Days 1, 4, and every two or three days thereafter until test end. Mortality was recorded on Days 1, 2, 7, and 9 after test start. Missing nymphs were considered dead. The test is considered valid if control mortality is <25%, and mortality in the standard reference control is around 40% (Bakker et al., 2000).

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin √x function and analyzed using the Kruskal-Wallis test (p≤0.05).

### Trial 6 Results

Results are summarized in Table 11. Mean mortality in the test material group was significantly higher than in the untreated control on Days 7 and 9.

<b>TABLE 11. Mean mortality (%) of <i>Orius laevigatus</i> fourth-stage nymphs exposed to Futureco NoFly</b>				
<b>Treatment</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 7</b>	<b>Day 9</b>
Untreated control	0 ± 0a	0 ± 0a	6.9 ± 2.78a	8.3 ± 2.41a
Futureco NoFly	0 ± 0a	0 ± 0a	45.8 ± 6.36c	75 ± 8.67b
Reference control	13.9 ± 5.01b	36.1 ± 6.94b	91.7 ± 2.41b	95.8 ± 2.41b
Selective reference control	0 ± 0a	0 ± 0a	9.7 ± 1.39a	19.4 ± 9.11a

Data from p. 19, MRID 49118305

Different letters within a column indicate significant differences ( $p \leq 0.05$ )

Corrected mortality is given in Table 12. The toxicity of the test material was classified as 3 (moderately dangerous) to the fourth stage nymph of *O. laevigatus* nine days after treatment, when mortality was 73.2%. According to the IOBC guidelines (Hassan et al, 1994; Bekker et al., 2000), a new trial under semi-field conditions must be conducted.

<b>TABLE 12. Toxicity of Futureco NoFly to <i>Orius laevigatus</i> fourth-stage nymphs</b>		
<b>Day</b>	<b>Corrected mortality (%)</b>	<b>Classification<sup>a</sup></b>
1	0 ± 0	1
2	0 ± 0	1
7	42.1 ± 5.29	2
9	73.2 ± 8.9	3

Data from p. 19, MRID 49118305

<sup>a</sup>According to IOBC/WPRS classification (Hassan et al., 1994, 2000)

### Trial 7

A study under semi-field conditions was conducted to determine the effects of contact exposure of the test material on mortality of first-stage nymphs of the whitefly predator *Orius laevigatus*. The test organisms were reared on bean pods at 25±1°C under 16 hrs light:8 hrs darkness. The test also included a toxic reference control group exposed to Deltamethrin 2.5 EC (0.04% in sterile tap water), a selective reference control group exposed to Atominal 10 EC (62.5 cm<sup>3</sup>/hL in sterile tap water), and an untreated control (sterile tap water only). The test material was applied at 5 x 10<sup>4</sup> cfu/cm<sup>2</sup> leaf.

The test organisms were exposed to the appropriate treatment on tomato plants (25 cm in height). The treatments were applied by spray until runoff. The plants were then covered with *Ephestia kuehniella* eggs and pollen as a food source. Five nymphs were placed on two full growth leaves of each plant. Each treatment consisted of four replicates of three plants each. Food was provided on Days 1 and 4. Temperature and humidity were recorded.

Mortality was recorded on Day 8. Missing nymphs were considered dead. For the test to be considered valid, mortality in the control should not exceed 25% and in the test material group should be lower than 40% (Bakker et al, 2000).

The test material was classified according to the following toxicity categories (Hassan et al. (1994):

<b>Scale</b>	<b>Denomination</b>	<b>Amended Mortality</b>
1	Innocuous	<25%
2	Slightly harmful	25-50%

3	Moderately harmful	50-75%
4	Harmful	>75%

The data were analyzed using SPSS 12.01 for Windows. Results for mean mortality in the replicates were transformed via the arcsin  $\sqrt{x}$  function and analyzed using the Kruskal-Wallis test ( $p \leq 0.05$ ).

### Trial 7 Results

During the test, the temperature ranged from 12.93 to 39.67°C, with mean of 21.41°C. The humidity ranged from 17.90 to 93.80%, with a mean of 59.74%.

Mean Day 8 mortality is summarized in Table 13. Mortality in the test material treatment was significantly higher than in the untreated control.

<b>TABLE 13. Mortality of <i>O. laevigatus</i> first-stage nymphs exposed to Futureco NoFly under semi-field conditions</b>	
<b>Treatment</b>	<b>Mortality (%) at day 8</b>
Untreated control	23.93 $\pm$ 1.92a
Futureco NoFly	41.70 $\pm$ 3.69b
Reference control	96.70 $\pm$ 1.92c
Selective reference control	46.70 $\pm$ 2.72b

Data from p. 22, MRID 49118305

Means with different letters are significantly different (Kruskal-Wallis,  $p \leq 0.05$ )

Corrected mortality is given in Table 14. Based on the corrected mortality, the test material was innocuous to the first stage nymph of *O. laevigatus* when applied at a rate of  $5 \times 10^4$  cfu/cm<sup>2</sup> leaf under semi-field conditions.

<b>TABLE 14. Toxicity of Futureco NoFly to <i>O. laevigatus</i> fourth stage nymphs</b>	
<b>Corrected mortality (%)</b>	<b>IOBC/WPRS classification</b>
23.9 $\pm$ 3.93	1

Data from p. 23, MRID 49118305

### Study Authors' Conclusions

The study authors concluded that under laboratory conditions, mortality and reduction of parasitism in the whitefly parasitic species *Encarsia formosa* and *Eretmocerus mundus* were unaffected by Futureco NoFly. Under laboratory conditions, a toxic effect of the test material was seen in the whitefly predatory species *Macrolophus caliginosus* and *Orius laevigatus*. However, under semi-field conditions, Futureco NoFly was innocuous to *O. laevigatus*. This was attributed to the possibility that the test material could develop and grow on the food source (eggs of *Ephestia kuehniella*), exposing *O. laevigatus* and *M. caliginosus* to dietary as well as contact exposure, producing unrealistic toxic effects. A slight toxic effect is possible to the predatory species.

### Reviewer's Conclusion

Based on the information provided, the reviewer agrees with the study authors' conclusions. Only summaries of the tests, conducted under IOBC guidelines, were provided in MRID 49118305. Neither a certificate of analysis nor confirmation of test material viability was provided, therefore

nominal doses cannot be verified. The individual studies should be submitted for review. As a result, this study is classified as supplemental.

The seven tests as reported appear scientifically valid, and the results are useful for evaluating short term exposures of the MPCA, *Paecilomyces fumosoroseus* Strain FE 9901 to nontarget insects, especially in the insect orders Hymenoptera, Homoptera and Hemiptera (i.e., parasitic wasps and predatory bugs.) Because toxic effects were noted in laboratory testing the predatory bugs, *Macrolophus caliginosus* and *Orius laevigatus* precautionary label language and/or additional nontarget arthropod testing to other beneficials may be required, depending on the use site(s), especially if IPM strategies are in development or in place.

TABLE 13. Mortality of *O. laevigatus* first-stage nymphs exposed to *Paecilomyces* NoFly under semi-field conditions

Treatment	Mortality (%) at day 8
Untreated control	13.93 ± 1.93
<i>Paecilomyces</i> NoFly	41.70 ± 3.60
Untreated control	56.70 ± 1.43
<i>Paecilomyces</i> NoFly	46.70 ± 2.73

Test Date: 22 MAR 2018  
Means with different letters are significantly different (Duncan-Wallis, p=0.05)

Controlled mortality is given in Table 14. Based on the controlled mortality, the test material was innocuous to the first stage nymph of *O. laevigatus* when applied at a rate of 2 x 10<sup>6</sup> cfu/cm<sup>2</sup> leaf under semi-field conditions.

TABLE 14. Toxicity of *Paecilomyces* NoFly to *O. laevigatus* first stage nymphs

Controlled mortality (%)	IOBC/WTR classification
25.9 ± 3.81	I

Test Date: 22 MAR 2018

## Study Authors' Conclusion

The study authors concluded that under laboratory conditions, mortality and reduction of population in the whitefly parasitic species *Encarsia formosa* and *Encarsia perniciosus* were unaffected by *Paecilomyces* NoFly. Under laboratory conditions, a toxic effect of the test material was seen in the whitefly predatory species *Macrolophus caliginosus* and *Orius laevigatus*. However, under semi-field conditions, *Paecilomyces* NoFly was innocuous to *O. laevigatus*. This was attributed to the possibility that the test material could develop and grow on the food source (eggs of *Ephestia kuehniella*), exposing *O. laevigatus* and *M. caliginosus* to dietary as well as contact exposure, producing unambiguous toxic effects. A slight toxic effect is possible in the predatory species.

## Reviewer's Conclusion

Based on the information provided, the reviewer agrees with the study authors' conclusion. Only summaries of the tests conducted under IOBC guidelines were provided in MRID 49118305. Neither a certificate of analysis nor confirmation of test material viability was provided, therefore

## **References**

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